

PCR 1 - linearise ADH1

| | | | | | | | | | | | | |
|---|---------------------------------|---|---------------------|--|----------------------|---|---|---------------------|--|--|--------------------------|---------------------|
| | PCR 20 µL reaction (units : uL) | | Final Concentration | | | PCR 20 µL reaction (uni Final Concentration | | | | | Plasmid dilution : | |
| 5X Phusion HF Buffer | | 4 | 1X | | | 5X Phusion GC Buffer | 4 | 1X | | | ADH1 : 1083 ng/ul | |
| 10 mM dNTPs | | 0.4 | 200 µM | | | 10 mM dNTPs | 0.4 | 200 µM | | | dilution 1:1000 | |
| 10 µM Forward Primer | | 1 | 0.5 µM | | | 10 µM Forward Primer | 1 | 0.5 µM | | | ADH1 dilué : 1ng/ui | |
| 10 µM Reverse Primer | | 1 | 0.5 µM | | | 10 µM Reverse Primer | 1 | 0.5 µM | | | 1ul to pipette in sample | |
| CMV-Ds-Red2 diluted (5ng/µL) | | 1 | 5 ng | | | CMV-Ds-Red2 diluted (5ng/µL) | 1 | 5 ng | | | | |
| DMSO (optional) | | 0.6 | 3 % | | | DMSO (optional) | 0.6 | 3 % | | | | |
| Phusion DNA Polymerase (add last) | | 0.2 | 1.0 units/50 µl PCR | | | Phusion DNA Polymerase (add last) | 0.2 | 1.0 units/50 µl PCR | | | | |
| Nuclease-free Water | | 11.8 | | | | Nuclease-free Water | 11.8 | | | | | |
| Total volume | | 20 | | | | Total volume | 20 | | | | | |
| | | | | | | | | | | | | |
| Number of reactions (including controls): | | 9 | | | | Number of reactions (including cont | 9 | | | | Forward Primer : | f_Gibs_ADH1 diluted |
| | | | | | | | | | | | Reverse Primer : | r_Gibs_ADH1 diluted |
| | | | | | | | | | | | | |
| WORK ON ICE | | | | | | WORK ON ICE | | | | | | |
| Mastermix | 5 reactions needed | | | | | Mastermix | 5 reactions needed | | | | | |
| 5X Phusion HF Buffer | | 36 | | | | 5X Phusion GC Buffer | 36 | | | | | |
| 10 mM dNTPs | | 3.6 | | | | 10 mM dNTPs | 3.6 | | | | | |
| 10 µM Forward primer | | 9 | | | | 10 µM Forward primer | 9 | | | | | |
| 10 µM Reverse primer | | 9 | | | | 10 µM Reverse primer | 9 | | | | | |
| DMSO (optional) | | 5.4 | | | | DMSO (optional) | 5.4 | | | | | |
| Phusion DNA Polymerase (ADDED LAST) | | 1.8 | | | | Phusion DNA Polymerase (ADDED | 1.8 | | | | | |
| Nuclease-free Water | | 115.2 | | | | Nuclease-free Water | 115.2 | | | | | |
| | | | | | | | | | | | | |
| Distribute 19 µl in tubes | | | | | | Distribute 19 µl in tubes | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Add the DNA (1µL) | Tube labelling | | | | | Add the DNA (1µL) | Tube labelling | | | | | |
| ADH1 | A | | | | | ADH1 | Ã | | | | | |
| ADH1 | B | | | | | ADH1 | ^B | | | | | |
| ADH1 | C | | | | | ADH1 | ^C | | | | | |
| ADH1 | D | | | | | ADH1 | ^D | | | | | |
| ADH1 | E | | | | | ADH1 | Ê | | | | | |
| ADH1 | F | | | | | ADH1 | ^F | | | | | |
| - | A- | | | | | - | ^A- | | | | | |
| - | B- | | | | | - | ^B- | | | | | |
| - | C- | | | | | - | ^C- | | | | | |
| - | D- | | | | | - | ^D- | | | | | |
| - | E- | | | | | - | ^E- | | | | | |
| - | F- | | | | | - | ^F- | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Centrifuge the tubes before the running the PCR | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| PCR programming | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Programme A : 'Y-ADH1-A' | | | | | | Programme B : 'Y-ADH1-B' | | | | | | |
| Initial Denaturation | 98°C | 30 seconds | | | Initial Denaturation | 98°C | 30 seconds | | | | | |
| | 98°C 61°C 72°C | 10 seconds 20 seconds 240 seconds | | | | 98°C 61°C 72°C | 10 seconds 20 seconds 180 seconds | | | | | |
| 35 Cycles | | | | | 35 Cycles | | | | | | | |
| Final Extension | 72°C | 10 minutes | | | Final Extension | 72°C | 10 minutes | | | | | |
| Hold | 4°C | | | | Hold | 4°C | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Programme C : 'Y-ADH1-C' | | | | | | Programme D : 'Y-ADH1-D' | | | | | | |

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|----------------------|----------------------|---|--|----------------------|----------------------|---|--|--|--|--|--|--|
| Initial Denaturation | 98°C | 30 seconds | | Initial Denaturation | 98°C | 30 seconds | | | | | | |
| 35 Cycles | 98°C 61°C 72°C | 10 seconds 30 seconds 210 seconds | | 35 Cycles | 98°C 59°C 72°C | 10 seconds 20 seconds 240 seconds | | | | | | |
| Final Extension | 72°C | 10 minutes | | Final Extension | 72°C | 10 minutes | | | | | | |
| Hold | 4°C | | | Hold | 4°C | | | | | | | |
| Programme 'Y-ADH1-E' | | | | Programme 'Y-ADH1-F' | | | | | | | | |
| Initial Denaturation | 98°C | 30 seconds | | Initial Denaturation | 98°C | 30 seconds | | | | | | |
| 35 Cycles | 98°C 59°C 72°C | 10 seconds 20 seconds 180 seconds | | 35 Cycles | 98°C 59°C 72°C | 10 seconds 30 seconds 210 seconds | | | | | | |
| Final Extension | 72°C | 10 minutes | | Final Extension | 72°C | 10 minutes | | | | | | |
| Hold | 4°C | | | Hold | 4°C | | | | | | | |